

Exosomes and Exosomal RNA

A Way of Cell-to-Cell Communication

Akademisk avhandling

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av

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Avhandlingen baseras på följande delarbeten:

- I. **Eldh M**, Lötvall J, Malmhäll C and Ekström K. **Importance of RNA isolation methods for analysis of exosomal RNA: Evaluation of different methods** *Mol Immunol.* 2012 Apr;50(4):278-86.
- II. **Eldh M**, Ekström K, Valadi H, Sjöstrand M, Olsson B, Jernås M and Lötvall J. **Exosomes Communicate Protective Messages during Oxidative Stress; Possible Role of Exosomal Shuttle RNA.** *PLoS One.* 2010 Dec 17;5(12):e15353.
- III. **Eldh M***, Olofsson R*, Lässer C, Svanvik J, Sjöstrand M, Mattsson J, Lindnér P, Choi DS, Gho YS and Lötvall J. **MicroRNA in exosomes released from liver metastases in patients with uveal melanoma.** *In manuscript.* * These authors contributed equally to this work.

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Abstract

Exosomes are nano-sized extracellular vesicles of endocytic origin participating in cell-to-cell communication, partly by the transfer of exosomal RNA between cells. These extracellular vesicles are released by most cells and found in many body fluids including plasma and urine. Exosomes differ compared to their donor cells in RNA, protein and lipid composition, and their molecular content has shown prognostic and diagnostic potential. Uveal melanoma is a tumour arising from melanocytes of the eye and despite successful control of the primary tumour, approximately one third of the patients will develop metastases, predominantly liver metastases, with poor prognosis. The overall aim of this thesis was to evaluate the role of exosomes in cell-to-cell communication and the biological role of exosomal RNA.

Exosomal RNA has been extracted by different RNA isolation methods and we identified that the RNA size distribution pattern varied in multiple studies. Therefore, we aimed to determine if this RNA variation was a true variation or merely a consequence of the RNA extraction method used. We evaluated seven different RNA isolation methods using a mouse mast cell line (MC/9) that continuously releases exosomes. The results showed that the exosomal RNA yield and size distribution pattern differed substantially between different RNA isolation methods.

The mRNA content and function of MC/9 cell-derived exosomes was shown to be altered depending on the culture conditions of the cells. Cells exposed to oxidative stress were shown to have the capacity to send a conditioning signal to other cells, resulting in resistance to oxidative stress in the recipient cells. Moreover, this conditioning signal was shown to be eradicated upon UV-C exposure, indicating a possible role for the exosomal RNA in this biological function.

The presence of exosomes in patients with liver metastases from uveal melanoma was established with the isolation, detection and characterisation of exosomes from isolated hepatic perfusion. The results revealed melanoma-specific exosomes, which contained similar microRNA profiles between patients. Furthermore, patients with metastatic uveal melanoma were shown to have a higher concentration of exosomes in their peripheral venous blood compared to healthy controls.

We conclude that exosomes play a role in cell-to-cell communication and their RNA appears to be of biological importance. Furthermore, exosomal RNA may potentially play a role in the diagnosis and prognosis of uveal melanoma.

Keywords: Exosomes, extracellular vesicles, RNA, mRNA, microRNA, RNA isolation, uveal melanoma

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